tion stabilization when transplanted into an established model of uremia (2-step 5/6 Nx), after onset of disease.

Example 20

Genetic Profiling of Therapeutically Relevant Renal Bioactive Cell Populations

[0587] To determine the unbiased genotypic composition of specific subpopulations of renal cells isolated and expanded from kidney tissue, gene array and quantitative real time PCR (qrtper) analyses (Brunskill et al., 2008) were employed to identify differential cell-type-specific and pathway-specific gene expression patterns among the cell subfractions.

[0588] The isolation and primary culture of unfractionated heterogeneous mixtures of renal cells has been described previously (Aboushwareb et al., 2008). Subsequently, standard density-gradient methodology was utilized to generate cell subfractions, which were then characterized based on specific biological activity and phenotypic characteristics. Ultimately, two specific subfractions, termed "B2" and "B4" were demonstrated to be of particular therapeutic value, alone and in combination, when transplanted intrarenally into a progressive model of CKD generated by a two-step 5/6 Nx procedure in female Lewis rats.

[0589] Cells and Cell Culture Conditions:

[0590] An established heterogeneous culture of male Lewis rat kidney cells was fractionated according to Example 8. Prior to gradient fractionation, the renal cells were cultured in 50:50 mixture of high glucose DMEM containing 5% (v/v) FBS, 2.5 μg EGF, 25 mg BPE (bovine pituitary extract), 1×ITS (insulin/transferrin/sodium selenite medium supplement), antibiotic/antimycotic (MFR) and cultured at 37° C. under standardized conditions of humidity and oxygen tension. The resulting subfractions (B1, B2, B3, B4, and pellet) were sampled to obtain RNA for expression analysis and then implanted into uremic rats to assess biologic function in vivo.

[0591] Materials and Methods:

[0592] Microarray platform: Affymatrix GeneChip Rat Genome 230 2.0 Array; Contract facility: Wake Forest University Health Sciences, Microarray Core Facility; Validation method: ABI/Invitrogen 7300 quantitative real time PCR (qrtper) analysis; RNA isolation: Qiagen RNA Isolation kit; cDNA synthesis: Invitrogen Vilo superscript cDNA isolation kit; Primers & probes: ABI/Invitrogen Taqman assays ('Inventoried' primers and probe sets)

[0593] Procedure:

[0594] Isolate and quantitate RNA from cell subfractions, immediately after subfractionation procedure (Table 24-25)

[0595] Affymetrix Gene array analysis on normalize (2 μg) RNA samples (data not shown)

[0596] Select differentially expressed genes based on p-value and fold change significance (data not shown)

[0597] Use David annotation assignment (http://david.abcc.ncifcrf.gov/) to categorize differentially expressed genes (data not shown)

[0598] Select genes to validate microarray by qrtper the specific subfractions, generated from a Lewis rat cell preparation, a normal human kidney cell preparation, and a human chronic kidney disease cell preparation. (Table 27)

TABLE 24

Culture conditions and gradient load.

Cell Prep	Seeding Density	Culture time	Final Confluency	Gradient Load
RK086	17.5 e^6/flask	3 d 21% O_2 1 d 2% O_2	100%	72.8 e^6
RK087	15 e^6/flask	2 d 21% O_2 1 d 2% O_2	85%	91 e^6
RK097	19.3 e^6/flask	2 d 21% O_2 1 d 2% O_2	85%	92.5 e^6

TABLE 25

RNA concentration and normalization.
RNA Normalization

		Fraction	Symbol	ng/ul5	Vol, 2 μg	Norm 20 μl
1	RK086	3812	PreG	412.19	4.852	15.148
2		3813	B1	511.62	3.909	16.091
3		3814	B2	460.28	4.345	15.655
4		3815	B3	284.08	7.040	12.960
5		3816	B4	163.64	12.222	7.778
6		3817	Pellet	354.38	5.644	14.356
7	RK087	3821	Macro	213.05	9.387	10.613
8		3825	PreG	301.08	6.643	13.357
9		3826	B1	363.74	5.498	14.502
10		3827	B2	351.53	5.689	14.311
11		3828	B3	370.35	5.400	14.600
12		3829	B4	387.13	5.166	14.834
13		3830	Pellet	136.67	14.634	5.366
14	RK097	4692	Macro	125.76	15.903	4.097
15		4697	PreG	379.67	5.268	14.732
16		4698	B1	366.56	5.456	14.544
17		4699	B2	420.82	4.753	15.247
18		4700	B3	439.3	4.553	15.447
19		4701	B4	350.43	5.707	14.293
20		4702	Pellet	167.94	11.909	8.091

[0599] Results:

[0600] Differential expression between fractions (B1-B4) and/or pre-gradient (PreG) was determined under the following stringent conditions: listed genes met both criteria of significance: p-value <0.05, and fold change <–0.5 or >0.5. Probe set IDs (ex.: 1395810_at | - - - | - - -) without a gene name/description correspond to gene array oligonucleotide (oligo) that has yet to be assigned. These oligos can be selected through the Affymetrix "Netaffx" web page https://www.affymetrix.com/analysis/netaffx and blasted against NCBI genomic databases to obtain a probability for gene assignment.

[0601] The summary of genes differentially expressed (Up/Down) between Pregradient and Post-gradient (B1-B4) cell populations is shown below in Table 26. The selection criteria for determining differences in gene expression: T-test pvalue ≦0.05 with an absolute fold change ≧0.5 between cell populations. For example, as shown in Table 26 below, the difference between Pre-gradient and B1: of the 165 differentially expressed genes, 32 were up in B1, and 133 were down in B1 from Pregradient. The genes that represent differences in expression between these cell populations were determined.